penn state iGEM
bacterial relay race

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What is iGEM?

The International Genetically Engineered Machines (iGEM) Competition is an annual undergraduate research competition hosted by MIT. The project aim is to develop Synthetic Biology through the creation of a *registry of parts*. Each part in the registry is an analyzed strain of DNA with several specific restriction sites at each end of the fragment. These strains can be anything from promoters to genes, allowing easy assembly and reassembly of these parts into *genetic circuits*. The 2006 jamboree consisted of 37 teams from 12 countries whom presented their findings.

Introduction

The bacterial relay race consists of a signal transfer between two sets of engineered bacteria to control motility. In the relay, sender cells swim towards a second set of immotile receiver cells, transferring a signal which activates motility in the latter cell group. The design utilizes inducible promoters involved in quorum sensing to activate the motility gene which consists of an essential protein in the flagellar machinery, without which there can be no movement.

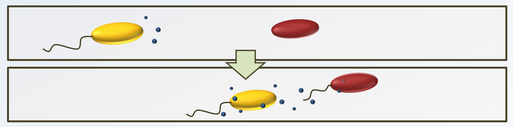


Figure 1: A sender cell approaches the receiver cell, where a quorum signal is transferred activating the motility in the latter set.

The creation of the highly sensitive bacterial relay race has several important applications. Most importantly, the relay offers a unique testing ground in which methods can be developed to deal with leaky expression (where certain genes are unintentionally expressed). Leaky expression continues to be a large problem in Synthetic Biology, and the application of methods employed in the relay race could prove helpful in other designs. Also, the inducible motility of the relay has potential in microfluidic applications such as chemical sensing and drug delivery devices.

Testing Strategy

Induce motility in Receiver Cells Chemically ☑

The receiver circuit was built and tested by adding HSL at various concentrations to ensure that motility could be sufficiently repressed and activated at low concentrations. *(data not shown)*

Induce Receiver with Sender Cells ☑

The receiver circuit was plated with an immotile sender on a swarm plate to ensure that output signal from sender cells could induce Receiver cells. (figures 6 & 7)

Induce Receiver within microchannel ☐

Placing the receiver within the microchannel, while the sender cells are allowed to swarm in inducing the Receivers

Interested in learning more about Penn State iGEM?
Visit http://openwetware.org/wiki/IGEM:PennState on the world wide web

Strategy

Cell Signaling

Small diffusible molecules known as quorum signals (figure 2) are constitutively expressed in the sender cells, and freely diffuse across the cell membrane. As the sender approaches the immotile receiver cell the quorum molecules enter the receiver and bind to constitutively expressed regulator protein LuxR, the activated molecule can then bind to the Lux promoter in the "Motility + Reporter circuit" resulting in the expression of MotB in the receiver cell.

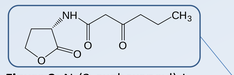
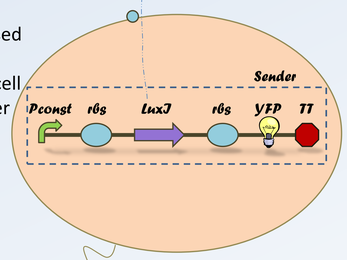


Figure 2: N-(3-oxohexanoyl)-L-homoserine lactone used to induce motility in receiver cells



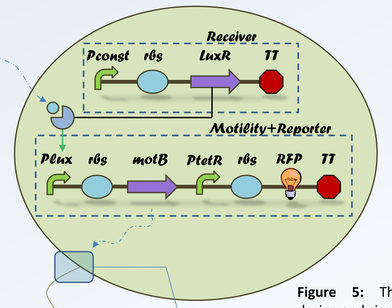


Figure 5: The circuit design and signal transfer between the sender *(left)* and receiver *(right)* cells.

Cell Signaling in Nature

In nature, *V. Fischeri* has been shown to use quorum sensing to control luminescence[3]. As shown in figure 3, at high densities the bacteria emit light, attracting their preferred hosts such as sepolid squid and moncentrid fish. Once within the host quorum sensing is used to optimize cell density.

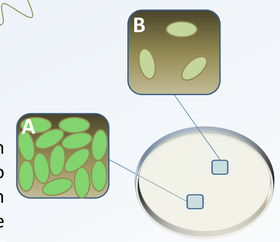


Figure 3: A cluster of cells in A expressing fluorescence, whereas cells in low density areas (B) do not have the quorum of molecules to express fluorescence.

Motility Control

A deletion of *motB* from the chromosome, with an inducible copy in the plasmid has been shown to allow restoration of motility in an average of 10 minutes[2].

MotB acts as a proton pump coupling proton motive force to torque generation, and is one of the last proteins in the flagellum to assemble (figure 4). Cells lacking the protein are unable to rotate their flagellum and therefore cannot swarm.

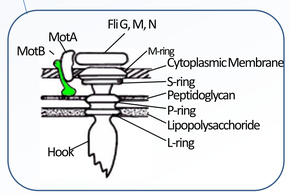


Figure 4: MotB couples proton motive force to torque generation and is required for the rotation of the flagellum. (*Modified Escherichia coli swim on the right-hand side)*

Results

In order to test the success of our circuit design, immotile sender cells are inoculated on an agar plate with receiver cells at various distances. Homoserine Lactone produced by the sender cells then diffuses allowing a restoration of motility within the receiver.

In one motility assay (figure 6), the receiver colony placed closest to the sender cells became motile, swarming with the diameter to be approximately double of the farther away colonies. A negative control with no sender cells to induce motility did show some level of motility. In an alternative setup, receiver cells were placed at various distances in an arching pattern away from the sender cell (figure 7). Receiver cells exhibited a level of motility inversely related to distance from sender.

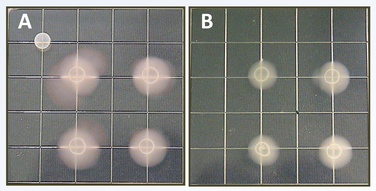


Figure 6: Plate A with sender cells (top left plated 6 hours) and Receiver Cells at various distances (colored red). Plate B with four Receiver Cell colonies and no Sender Cells. Plates: .30% Eiken Agar plates incubated at 30°C *(pictures taken 19 hours after inoculation).*

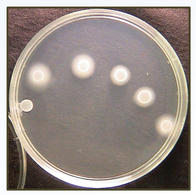


Figure 7: Receiver colonies placed at various distances from sender colony (left). Plates: .30% Eiken Agar plates incubated at 30°C *(pictures taken 20 hours after inoculation).*

Conclusions

Significant progress had been made towards full control of motility expression using cell to cell signaling. Receiver cells have been induced by a group of sender cells at various distances, resulting in varying levels of motility expression within the receiver cells which is inversely related to the distance from the sender cells.

Leaky expression of *motB* continues to be problematic in noninduced cells, although the induced cells appeared significantly more motile. Some procedural changes such as lower incubation temperatures combined with higher concentration agar may help in resolving this problem.

Attempting to repress *motB* more strongly may effect the ability of the receiver cell to become induced in the microchannel where quorum signal concentrations will likely be much smaller than the plate conditions. Using external variables such as plate composition and temperature may offer clues as to how to adjust similar circuits to eliminate or greatly reduce background expression.

The bacterial relay race like many other projects in synthetic biology work to advance the field through part characterization, and increasing the understanding of specific part interactions.

Microchannels

Microchannels offer a superb environment to host the bacterial relay race. The constrained channel volume increases quorum signal levels while resolving issues of directional movement. This Is due to the rotational nature of the flagellum, where swimming cells will Move against the right wall of a micro-channel without changing directions[1]. Figure 8 shows this phenomenon. The microchannels are made from a plasma treated PDMS mold of a nanofabricated wafer, and placed feature side down into a .30% eiken agar swarm plate.

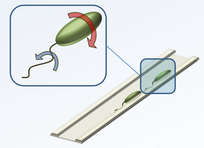


Figure 8: Rotation of the flagellum causes torque in the opposite direction on the cell body, keeping the cell to the right side of the channel.

Future Work

Address leaky expression of *motB* within the receiver cell.

Construct and implement feedback loop from receiver to sender cells to discontinue sender cell motility after signal transfer (figure 9).

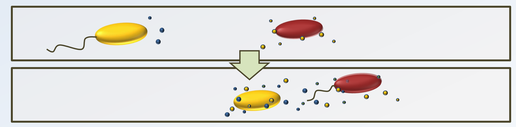


Figure 9: A motile cell type approaches the immotile cell, where a quorum signal is transferred activating the motility in the latter set, while a second signal is received by the first cell disabling motility.

Optimize channel for cell interaction and delivery.

Experiment with alternative microchannel geometries (figure 10).

Figure 10: A circular track offers an opportunity for cells to participate more than once within the relay race.

Generalize cell types such that two cells types could be used in a repeated fashion to expand the relay race to more than two cells.

Work Cited

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2) DiLuzio, W.R. *et al.: 'Escherichia coli swim on the right-hand side', nature 2005, 435,* pp. 1271-1274
3) Kaiser, D., Losick, R. 'How and Why Bacteria Talk to Each Other,' Cell 1993, 73, pp. 873-85

Acknowledgments

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